bio-protocol Preprint

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Adapter ligation and RT-PCR

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An abbreviated version of this protocol was published in eLIFE in Feb 2022

Rolling circle RNA synthesis catalyzed by RNA

DOI: 10.7554/eLife.75186

Detailed protocol

Detailed protocol for "Adapter ligation and RT-PCR"

For paper: Rolling circle RNA synthesis catalyzed by RNA, eLife 2022 (https://doi.org/10.7554/eLife.75186)

Adding 3'-end adapter to primer-extended RNA samples:

Initially primer extension reactions are run using F8+Adap or F9+Adap RNA primers (see sequence in Supp. File 1 – oligonucleotide sequences). After Urea PAGE extracting the RNA products - by cutting out the appropriate area of the gel - RNA is pelleted in 0.5 mL tube (with glucagon carrier).

Pelleted RNA is dissolved in:

4.30 µL	Water
1 µL	(x10) T4RNA ligase buffer (no ATP) (NEB), (50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, and 1 mM DTT)
3 µL	50% PEG800
0.2 µL	2% Tween20
0.5 µL	adenylated *Adap1* DNA (10 µM) (see sequence in Supp. File 1 – oligonucleotide sequences), *
1 µL	T4 RNA ligase2 truncated (K227Q) (NEB)

10 µL total volume

Incubate at 16 ^{O}C for 2 hours

* for making adenylated DNA we followed the Protocol for Oligonucleotide Adenylation (E2610, NEB) with the mth RNA Ligase enzyme also supplied by NEB. Adenylated DNA was Urea-PAGE purified before further use.

Combined Reverse transcription and PCR (RT-PCR) of adapter-ligated RNA:

10 µL of adapter-ligated RNA was mixed into:
37 µL water
0.5 µL PCRp3 (100 µM)
0.5 µL RTp1 (100 µM)
50 µL 2x master mix (Thermo Fisher Scientific)
2 µL SSIII Taq mix (SuperScript III One-Step RT-PCR System with Platinum Taq DNA polymerase, Thermo Fisher Scientific)
100 µL total volume

RT-PCR program:

1. Reverse transcribing:	60 ^{O}C	for 30 minutes
2. Heating	94 ^{O}C	for 5 min
3. Melting:	94 ^{O}C	for 15 sec
4. Annealing:	50 ^{O}C	for 15 sec
5. Elongation:	68 ^{O}C	for 15 sec
6. Final elongation	68 ^{O}C	for 2 min
7. Store	5 ^{O}C	forever.

Repeat step 3 to 5 X times.
(Use as few cycles (X) as possible before correcting bands are seen in a 3% agarose gel, 12 cycles should do it but as much as 25 cycles or more might be needed before seeing bands from very low concentrations of the original RNA).
Analyze RT-PCR with 3% agarose gel stained with ethidium bromide or equal.

50 µL is loaded to an ethidium bromide stained 3% agarose gel electroporated and correct bands are cut-out and cleaned up by QIAGEN gel extraction kit (QIAGEN, Hilden, Germany).

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Kristoffersen, E. , Noy, A. and Holliger, P. (2022). Adapter ligation and RT-PCR. Bio-protocol Preprint. bio-protocol.org/prep1894.
2. Kristoffersen, E. L., Burman, M., Noy, A. and Holliger, P.(2022). Rolling circle RNA synthesis catalyzed by RNA. eLIFE. DOI: 10.7554/eLife.75186